

Fractional Withdrawal of Complement and Amboceptor by Means of Antigen. (Preliminary Note.)

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The problem which forms the objective of the present investigation is the determination of the extent to which complement and amboceptor are withdrawn by amounts of antigen which are insufficient to cause complete removal of these two substances.

In this investigation the source of both complement and amboceptor was normal human blood serum, which was employed alone when it was desired to preserve the natural relationship of complement and amboceptor; when it was desired to vary this relation, artificial mixtures of complement and amboceptor were prepared, which presented wide divergence from the relation obtaining in normal serum.

Human serum has a hæmolytic action, not very considerable in degree, upon the red blood cells of the rabbit, which were employed as antigen. In all experiments made with this hæmolytic system the same volume of fluid was employed. Estimations of complement and amboceptor were expressed in terms of the equivalent quantities of active and inactivated normal serum respectively, it being found that the composition of healthy sera ordinarily exhibited only slight variation, any marked change in the content of these two substances being exceptional.

The estimation of complement was sometimes made by determining the maximum amount of red blood cells completely laked by a given quantity of complement-holding fluid in the presence of a considerable excess of amboceptor, and then ascertaining the amount of normal serum capable of producing the same degree of hæmolysis under similar conditions of experiment. It was, however, sometimes found more convenient to keep the amount of red blood cells employed constant, and to vary the conditions of experiment until these were so adjusted that complete hæmolysis was observed at the end of a period of 90 minutes at a temperature of 37° C.

The estimation of the amount of amboceptor in serum was carried out by first determining the amount of complement present and then ascertaining, in the absence of any addition of amboceptor, the hæmolytic power

of the serum. The latter was then compared with the hæmolytic power of a number of admixtures of complement and amboceptor, in which the content of complement was the same as in the serum tested, but the amounts of amboceptor present formed an increasing series, the comparison being made under similar conditions of experiment. The quantity of amboceptor is represented by the amount contained in that admixture which exhibited the same hæmolytic power as the serum under consideration.

Before determining the extent to which partial withdrawal of complement and amboceptor by subminimal amounts of antigen occurs, it was necessary to study the effect of variations in the amounts of complement and amboceptor in respect of hæmolytic power. An investigation involving this inquiry has been carried out with the aid of a heteroamboceptor by Kiss,* who determined the varying amounts of complement and amboceptor which were just capable of producing complete hæmolysis of the same amount of red blood cells. This author observed that as the complement was increased in amount the quantity of amboceptor required became diminished. In our own experiments, in which the employment of a heteroamboceptor was avoided, both complement and amboceptor being derived, as already mentioned, from the same source, a similar relationship is also exhibited in a quantitative form. When a normal serum is employed the extent to which a disproportion of complement and amboceptor can be produced is limited by the relative feebleness of the serum. If, however, an immune serum is used, wider divergences in the amount of complement and amboceptor employed may be obtained.

If, in normal serum, fractional withdrawal of complement and amboceptor by means of antigen is carried out, it is found that these two substances are withdrawn in the proportion in which they naturally exist in the serum, so that the liquid remaining after partial withdrawal has been effected is comparable, as far as complement and amboceptor are concerned, to diluted normal serum.

If, instead of employing normal serum alone, a mixture of normal active serum (complement + amboceptor) with inactivated normal serum (amboceptor) is used, so that a liquid is obtained in which the concentration of amboceptor is increased, while, at the same time, the concentration of complement is reduced, it is found that complement and amboceptor are at first removed by subminimal amounts of antigen in approximately the same relative proportion in which they exist in the mixed sera, the amount of amboceptor being,

* "Untersuchungen über die Fermentnatur des Komplementes," 'Zeitschr. f. Immunitätsforschung,' 1909, vol. 3, p. 558.

relatively to the complement, removed in excess. If this relationship is studied more closely it is seen that the actual quantity of amboceptor removed is greater than that withdrawn by the same amount of antigen from a similar concentration of amboceptor afforded by active normal serum, while the actual quantity of complement removed is less than would be withdrawn had active normal serum been employed.

If normal serum from which amboceptor has been removed by means of antigen acting at 0° C. is added to inactivated serum (amboceptor), so that a mixture is produced in which complement is in excess, it is found that fractional withdrawal of complement and amboceptor proceeds in a similar manner, relatively more complement than amboceptor being removed. If the actual quantities withdrawn are considered, it is found that the amount of complement taken up by a given amount of antigen is greater than, and the amount of amboceptor less than, would have been removed had active normal serum been employed to produce the same concentration of amboceptor.

The mode of fractional withdrawal of complement and amboceptor by antigen is not such as would be expected if a simple stoichiometric relation between these three substances existed. Whatever the ultimate nature of the reaction in question may be, in the early stages of the process, at any rate, no fixed relationship between the quantities interacting exists.

When fractional removal of complement and amboceptor takes place in an artificial admixture of these two substances, the relation between the complement and amboceptor removed approximates at first to that obtaining in the admixture, provided the amounts removed are small. If relatively large amounts of complement and amboceptor are removed by antigen, the divergence between the two, though still present, tends to become less marked. In consequence, the difference between the complement and amboceptor still remaining becomes less pronounced, and, as withdrawal proceeds, tends to approach the relationship obtaining in normal serum. By using in these experiments complement and amboceptor contained in the same serum and thus avoiding the employment of a hetero-complement (by which is meant a complement which is heterologous in respect of the amboceptor employed), it becomes possible to compare the relation of complement and amboceptor in the liquid remaining after fractional withdrawal of these substances from an admixture with that obtaining in normal serum. It is seen that, as withdrawal proceeds, the liquid remaining tends to become more and more closely comparable, in respect of complement and amboceptor, with diluted normal serum.